MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF TALC

Talc (MP 10-52 Grade) was obtained from Walsh and Associates (North Kansas City, MO) in two lots (lot numbers W101882 and B5415). The talc purchased was manufactured by the Minerals, Pigments, and Metals Division of Pfizer, Inc. and is one of their microtalc series of products. Both lots were from Pfizer's Barretts, Montana, mine which is a strip mine located between Barretts and Three Brother, Montana. This mine is the only source for the MP 10-52 grade talc. The grade designation is for high purity talc that has a top particle size of 10 μm and according to the manufacturer contains no tremolite or any asbestiform minerals. Lot W101882 was used from the beginning of the 2-year studies through January 1986. Lot B5415 was used in the 2-year studies from 27 January 1986 to the end of the studies on 31 October 1986. The talc was extensively characterized by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and by McCrone Associates (Norcross, GA). The methods and results of these studies are detailed in Appendix H.

The study mineral, a finely powdered white solid, was identified as talc by infrared spectroscopy, elemental analysis, Karl Fischer water analysis, thermogravimetric analyses, spark source mass spectrometry, automated scanning electron probe analyses, X-ray diffraction, polarized light microscopy, and transmission electron microscopy. Both lots were shown to be asbestos free by polarized light microscopy and transmission electron microscopy. Results of automated scanning electron microprobe analysis of lot W101882 indicated that the sample was virtually free of silica (1 particle of silica in 1,466 particles examined). Bulk chemical stability studies were not conducted due to the physical and chemical properties of talc. During the study the compound was stored in tightly sealed plastic bags at 25° C.

GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS

Talc aerosols were generated in a single fluidized-bed generator (FBG) by injecting compressed air into the bed (Figure H2). The aerosolized talc particles were then mixed with diluting air before being delivered to the exposure chambers (Hazelton 1000 and 2000, Lab Products, Inc.). A second FBG for the control chamber contained only the stainless steel bed material (Figures H3 and H4).

Aerosol concentrations were monitored each day in each chamber by taking three, 2-hour filter samples. Background concentrations of suspended particles were measured each day in the control chamber by taking a 6-hour filter sample. A RAM-S forward light scattering monitor (GCA, Bedford, MA) was used to determine the stability of the aerosol concentrations and the need to adjust the aerosol generation system during the exposure. Determinations were made at the beginning, middle, and end of each filter sampling period. The overall mean concentrations were 5.9 and 16.7 mg/m^3 for the mouse study and 6.1 and 18.6 mg/m^3 for the rat study. While the overall means were very close to target concentrations, there were problems experienced in maintaining control of chamber concentrations. Weekly mean exposure concentrations for the 2-year studies are presented in Figures H5 through H8.

Chamber Atmosphere Characterization

Uniformity of the aerosol concentrations in each chamber was determined at approximately 3-month intervals with the RAM-S. The spatial variation as estimated by the relative standard deviation (RSD) was higher in the mouse study than the rat study with values ranging from 12% to 44% (RSD) for the mice and 2% to 31% (RSD) for the rats. To minimize the variation in talc concentrations, the animal cages were rotated once each week.

The time to reach 90% of the target concentration (T_{90}) was approximately 10 minutes. Therefore, the length of the exposure was defined at 6 hours plus the T_{90} of 10 minutes.

The aerosol size distribution was determined once each month for each chamber using a cascade impactor. The average mass mean aerodynamic diameter (MMAD) and the geometric standard deviation (σ_g) were calculated to be 3.3 ± 1.9 μm and 3.6 ± 2.0 μm for the 6 and 18 mg/m^3 mouse

chambers. The values were 2.7 ± 1.9 μm and 3.2 ± 1.9 μm for the 6 and 18 mg/m³ rat chambers. The individual values are presented in Tables H1 and H2.

Study Design

Groups of 50 male and 50 female rats and mice were selected for whole body inhalation to talc at target concentrations of 0 (chamber controls), 6, or 18 mg/m³. Rats were exposed for 6 hours daily, 5 days a week until mortality in any exposure group reached 80% (113 weeks for males and 122 weeks for females). Exposure of rats to talc was extended beyond 2 years based on the report that 80% of pulmonary neoplasms induced in rats by inhalation exposure to diesel exhaust occurred after 2 years (Mauderly *et al.*, 1986). Mice were exposed for 103 or 104 weeks. At the conclusion of the exposures, rats were exposed to filtered air for 10 or 11 days, while mice were exposed to filtered air for 10 to 14 days. All animals were subjected to necropsy and a complete pathology evaluation.

Additional special study groups of 22 male and 22 female rats and 40 male and 40 female mice similarly exposed to 0, 6, or 18 mg/m³ were designated for interim pathology evaluations; lung talc burden measurements; serial pulmonary function measurements (rats only); and lung biochemistry, cytology, and phagocytosis measurements. Rats were evaluated at 6, 11, 18, and 24 months, while mice were evaluated at 6, 12, and 18 months. Insufficient numbers of rats remained alive at week 103 of exposure for both pulmonary function and/or lung biochemistry/cytology and pathology distribution groups, therefore the remaining rats in these groups were combined. The numbers of rats and mice evaluated for pulmonary function and lung biochemistry, cytology, and phagocytosis and the methods used for each of the parameters are presented in Appendix F for rats and Appendix G for mice.

Source and Specification of Animals

Male and female F344/N rats were obtained from Lovelace Inhalation Toxicology Research Institute (Albuquerque, NM). Male and female B6C3F$_1$ mice were obtained from Frederick Cancer Research Center (Frederick, MD). Rats and mice were held 3 weeks before the studies began. Rats were 6 to 7 weeks old, and mice were 7 weeks old, when the studies began. Animal health was monitored by serologic analyses during the studies under the protocols of the NTP Sentinel Animal Program.

Animal Maintenance

Rats and mice were housed individually throughout the studies. Drinking water was available *ad libitum*. Further details of animal maintenance are given in Table 1.

Clinical Examinations and Pathology

All rats and mice were observed twice daily. Clinical observations and body weights were recorded at the beginning of the studies, weekly for 13 weeks, and monthly thereafter.

A necropsy was performed on all rats in the lifetime core study and all mice in the 2-year core study. Organ weights were recorded for the brain, heart, right kidney, liver, and lungs at the end of the studies. During necropsy, all organs and tissues were examined for grossly visible lesions. A complete histopathologic examination was performed on all animals. Tissues for microscopic examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned to a thickness of 5 μm, and stained with hematoxylin and eosin.

Microscopic evaluations were completed by the study laboratory pathologist and the pathology data were entered into the Toxicology Data Management System (TDMS). The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit for accuracy of labeling and animal identification and for thoroughness of tissue trimming. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. A quality assessment pathologist reviewed lung and bronchial and mediastinal lymph nodes in rats and mice and nose in male mice for accuracy and consistency of lesion diagnosis.

The quality assessment report and slides were submitted to the Pathology Working Group (PWG) chair, who reviewed tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. All pulmonary neoplasms in female rats and representative histopathology slides of adrenal gland (rats), bronchial lymph node, lung, mediastinal lymph node (rats), and nose, or lesions of general interest were presented by the chair to the PWG for review. The PWG included the quality assessment pathologist as

well as other pathologists experienced in rodent toxicologic pathology who examined these tissues without knowledge of dose group or previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the final diagnosis was changed to reflect the opinion of the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analysis of pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the Results section of this report. Animals were censored from the survival analyses at the time they were found dead from other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table tests to identify dose-related trends. All reported P values for the survival analysis are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 are given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of all nonneoplastic lesions and most neoplasms (Tables A2, B2, C2, and D2) are also given as the ratio of the number of affected animals to the number of animals with the site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., skin, intestine, harderian gland, and mammary gland) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Neoplasm Incidences

The majority of tumors in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The dosed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When tumors are incidental, this comparison of the time-specific tumor prevalences also provides a comparison of the time-specific tumor incidences (McKnight and Crowley, 1984).

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal tumors, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of tumor-bearing animals.

Tests of significance include pairwise comparisons of each dosed group with controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of tumor incidence, and reported P values are one sided. The procedures described above also were used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, see Haseman (1984).

Analysis on Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test was used, a procedure based on the overall proportion of affected animals.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data that had

approximately normal distributions were analyzed using the parametric multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955). Lung burden parameters that had skewed distributions were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response trend (Dunnett's or Dunn's test).

Quality Assurance Methods

The lifetime and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as study records were submitted to the NTP Archives, they were audited by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and preliminary review draft of this NTP Technical Report were conducted. Audit procedures are presented in the reports, which are on file at the NIEHS. The audit findings were reviewed and assessed by the NTP staff so that all discrepancies had been resolved or were otherwise addressed during the preparation of this Technical Report.

TABLE 1
Experimental Design and Materials and Methods in the Lifetime and 2-Year Inhalation Studies of Talc

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Study Laboratory
Lovelace Inhalation Toxicology Research Institute (Albuquerque, NM)

Strain and Species
Rats: F344/N
Mice: $B6C3F_1$

Animal Source
Rats: Lovelace Inhalation Toxicology Research Institute (Albuquerque, NM)
Mice: Frederick Cancer Research Center (Frederick, MD)

Time Held Before Studies
3 weeks

Average Age When Placed on Studies
6-7 weeks

Date of First Exposure
Rats: 2 July 1984
Mice: 4 June 1984

Duration of Exposure
Rats: 6 hours/day, 5 days/week for 113 weeks (males) and 122 weeks (females)
Mice: 6 hours/day, 5 days/week for 103-104 weeks

Date of Last Exposure
Rats: 29 August 1986 (males) and 31 October 1986 (females)
Mice: 30 May 1986

Average Age When Killed
Rats: 120-121 weeks (males) and 129-130 weeks (females)
Mice: 110-112 weeks

Method of Sacrifice
Injection of T-61 solution for all rats in the lifetime study, all rats designated for pathologic evaluation, and all mice. Halothane anesthesia for all rats designated for biochemical interim evaluations.

Necropsy Dates
Rats: 8-9 September 1986 (males) and 10-11 November 1986 (females)
Mice: 9-13 June 1986 (males) and 2-6 June 1986 (females)

Size of Study Groups
50 males and 50 females

Method of Animal Distribution
Assigned to groups by weight and sex using computer-generated random numbers.

Animals per Cage
1

Method of Animal Identification
Toe clip and ear tag

Diet
NIH-07 Rat and Mouse Ration (Zeigler Bros., Gardner, PA) available *ad libitum* during nonexposure periods

Maximum Storage Time for Feed
90 days

TABLE 1
Experimental Design and Materials and Methods in the Lifetime and 2-Year Inhalation Studies of Talc
(continued)

Water
Automatic Watering System (Edstrom), available *ad libitum*

Cages
Stainless steel mesh cages (Hazleton, Aberdeen, MD)

Chambers
Rats: Stainless steel multitiered whole-body exposure chambers (H2000, Hazleton Systems, Aberdeen, MD), washed once weekly
Mice: Stainless steel multitiered whole-body exposure chambers (H1000, Hazleton Systems, Aberdeen, MD), washed once weekly

Bedding
Untreated paper cage board (Shepherd Specialties Paper, Inc., Kalamazoo, MI), changed twice a day

Filters
Room Air and Chamber Air High Efficiency Particulate Air (HEPA) Filter (prefilter and exit filter), MIL Spec MIL-F-51068C (Flanders, Washington, DC)

Animal Room Environment

Rats	Mice
Average temperature: 24° C	Average temperature: 24° C
Relative humidity: 9%-100%	Relative humidity: 10%-100%
Fluorescent light: 12 hours/day	Fluorescent light: 12 hours/day
Room air changes: minimum of 10 changes/hour	Room air changes: minimum of 10 changes/hour

Exposure Concentrations
0, 6, and 18 mg/m^3 by inhalation

Type and Frequency of Observation
Observed twice daily; body weights and clinical findings recorded at study initiation, weekly through week 13, and monthly thereafter

Necropsy
Necropsy performed on all animals. Organ weights recorded for brain, heart, right kidney, liver, and lung.

Histopathology
Complete histopathologic examinations performed on all animals. In addition to tissue masses and gross lesions, tissues examined included: adrenal gland, bone (including marrow), brain, clitoral gland (female rats), epididymis, esophagus, gallbladder (mice), harderian gland (female rats and mice), heart, kidney, large intestine (cecum, colon, rectum), larynx, liver, lung, lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland (male rats), prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, ileum, jejunum), spleen, stomach (forestomach, glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.

RESULTS

RATS

4-WEEK STUDY DOSE SELECTION

Selection of 6 or 18 mg talc/m^3 as the exposure concentrations was based on the results of a 4-week inhalation study in F344/N rats to determine lung talc burden and histopathologic changes associated with talc exposure. These studies indicated that the amount of talc retained in the lung was similar between sexes and proportional to exposure concentration (Appendix K). Microscopic examination of the lungs revealed an accumulation of alveolar macrophages in the lungs only at the 18 mg/m^3 concentration. Based on these findings it was predicted that aerosol concentrations greater than 18 mg/m^3 would overwhelm lung clearance mechanisms, impair lung function, and possibly shorten survival.

LIFETIME STUDY

Survival

Estimates of survival probabilities for male and female rats are shown in Table 2 and in the Kaplan-Meier curves in Figure 1. Survival of exposed male and female rats was similar to that of the controls.

Body Weights and Clinical Findings

The mean body weights of male and female rats exposed to 6 mg/m^3 talc were similar to those of controls throughout the study (Tables 3 and 4, and Figure 2). Mean body weights of male and female rats exposed to 18 mg/m^3 were slightly lower than those of controls, particularly after week 65. The final mean body weight of males in the 18 mg/m^3 group was 4% lower than that of the controls, while the final mean body weight of females in the 18 mg/m^3 group was 14% lower than that of the controls.

Serological tests were performed prior to the beginning of the study and after 6, 12, and 18 months of exposure; serological tests were negative for all microorganisms tested (Table J1). After 24 months and 28 and 30 months (females), the serological tests were positive for Kilham rat virus (KRV), Sendai virus, and rat coronavirus/sialodacryoadenitis virus (RCV/SDA). The significance of the positive KRV titer is unknown since it was found in only one rat and was not observed at later times. No clinical findings or gross or microscopic lesions that could be attributed to Sendai virus or RCV/SDA infections were observed in the talc exposed or control groups. Since there was no clinical or pathological evidence of disease and since the infection occurred very late in the study, these subclinical infections are believed to have had no impact on the study results.

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplastic or nonneoplastic lesions of the lung, lymph node, nose, and adrenal medulla. Summaries of the incidences of nonneoplastic lesions and neoplasms, the individual animal neoplasm diagnoses, and the statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one group are presented in Appendix A for male rats and Appendix B for female rats.

TABLE 2
Survival of Rats in the Lifetime Inhalation Study of Talc

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
Lifetime Study Groups			
Animals initially in study	50	50	50
Natural deaths	18	17	14
Moribund kills	23	19	20
Animals surviving to study termination	9	14	16
Percent survival at end of study[a]	18	28	32
Mean survival (days)[b]	696	707	711
Survival analysis[c]	P=0.217N	P=0.422N	P=0.192N
Special Study Groups[d]			
Animals initially in study	22	22	22
Natural deaths	2	2	6
Moribund kills	9	5	6
Scheduled sacrifice	11	15	10
Females			
Lifetime Study Groups			
Animals initially in study	50	50	50
Natural deaths	11	19	14
Moribund kills	28	17	27
Missing[d]	0	1	0
Animals surviving to study termination	11	13	9
Percent survival at end of study[a]	22	28	18
Mean survival (days)[b]	743	753	758
Survival analysis[c]	P=0.846	P=0.805N	P=0.977
Special Study Groups[d]			
Animals initially in study	22	22	22
Natural deaths	2	1	2
Moribund kills	5	3	8
Scheduled sacrifice	15	18	12

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or lower mortality in a dose group is indicated by N.
[d] Censored from survival analyses

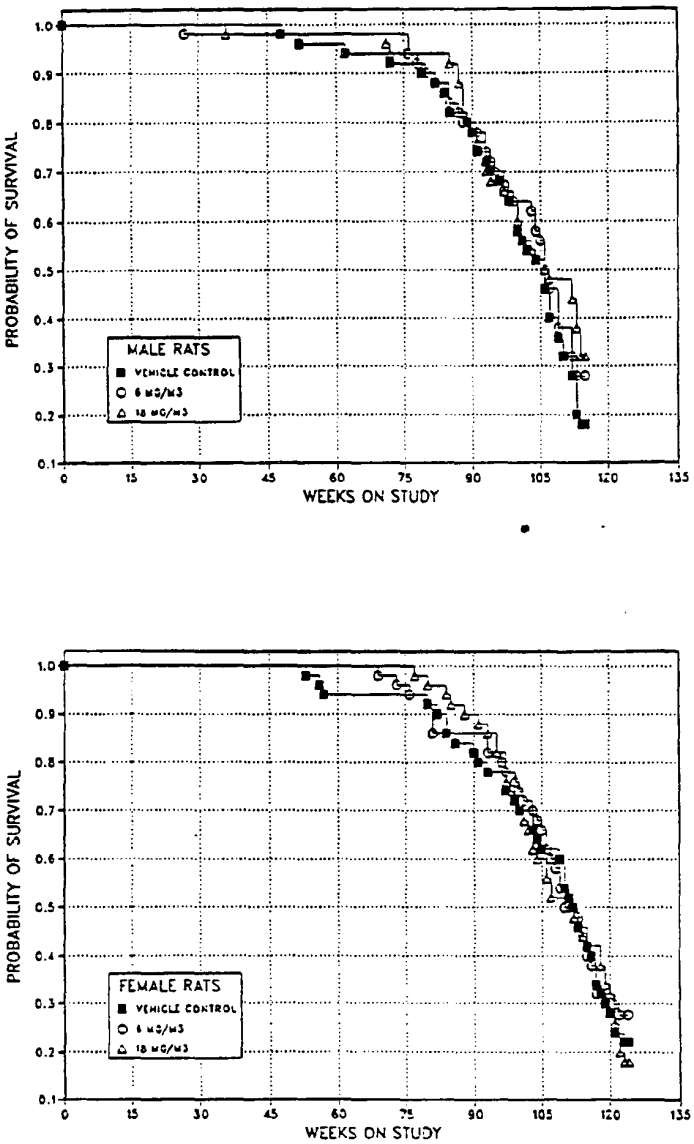

FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Administered Talc by Inhalation for Their Lifetime

TABLE 3
Mean Body Weights and Survival of Male Rats in the Lifetime Inhalation Study of Talc

Weeks on Study	0 mg/m³ Av. Wt. (g)	0 mg/m³ Number of Survivors	6 mg/m³ Av. Wt. (g)	6 mg/m³ Wt. (% of controls)	6 mg/m³ Number of Survivors	18 mg/m³ Av. Wt. (g)	18 mg/m³ Wt. (% of controls)	18 mg/m³ Number of Survivors
1	118	72	121	103	72	119	101	72
2	174	72	174	100	72	174	100	72
3	201	72	200	100	72	202	101	72
4	225	72	215	95	72	219	97	72
5	237	72	239	101	72	238	101	72
6	250	72	252	101	72	251	100	72
7	265	72	263	99	72	263	99	72
8	275	72	270	98	72	269	98	72
9	287	72	280	98	72	281	98	72
10	297	72	293	99	72	293	99	72
11	304	72	300	99	72	297	98	72
13	317	72	315	100	72	312	98	72
17	339	72	338	100	72	331	98	72
21	359	72	355	99	72	351	98	72
25	374	71	370	99	72	367	98	72
29[a]	380	68	378	99	68	369	97	69
33	398	68	393	99	68	386	97	69
38	407	68	405	100	68	393	97	68
41	413	68	412	100	68	401	97	68
45	421	68	420	100	68	410	97	68
49[a]	431	63	428	99	65	418	97	65
53	434	62	432	100	65	422	97	65
57	435	62	432	99	65	424	97	65
61	443	62	442	100	65	430	97	65
65	450	61	444	99	65	432	96	65
69	448	61	440	98	65	429	96	65
73	453	60	442	98	65	432	95	63
77	452	60	441	98	63	429	95	62
81[a]	444	55	434	98	57	423	95	59
85	450	49	434	97	53	424	94	57
89	447	47	437	98	50	424	95	51
93	434	43	429	99	48	408	94	46
97	429	40	427	100	41	407	95	40
101	410	34	395	96	40	394	96	34
105[a]	390	29	391	100	35	385	99	28
109	377	18	390	104	19	376	100	24
113	358	11	389	109	15	342	96	21
Terminal sacrifice		9			14			16
Mean for weeks								
1-13	246		244	99		243	99	
14-52	391		389	99		381	97	
53-113	428		425	99		411	96	

[a] Interim evaluations occurred during weeks 27, 47, 79, and 105.

TABLE 4
Mean Body Weights and Survival of Female Rats in the Lifetime Inhalation Study of Talc

Weeks on Study	0 mg/m³ Av. Wt. (g)	0 mg/m³ Number of Survivors	6 mg/m³ Av. Wt. (g)	6 mg/m³ Wt. (% of controls)	6 mg/m³ Number of Survivors	18 mg/m³ Av. Wt. (g)	18 mg/m³ Wt. (% of controls)	18 mg/m³ Number of Survivors
1	97	72	101	104	72	98	101	72
2	126	72	127	101	72	125	99	72
3	136	72	139	102	72	138	101	72
4	149	72	144	97	72[a]	145	97	72
5	153	72	159	104	72	154	100	72
6	160	72	165	103	72	160	101	72
7	165	72	169	102	72	166	101	72
8	168	72	171	102	72	168	100	72
9	174	72	176	101	72	173	100	72
10	178	72	182	102	72	179	101	72
11	181	72	184	102	72	181	100	72
13	186	72	191	103	72	187	101	72
17	194	72	201	104	72	197	101	72
21	206	72	211	103	72	207	101	72
25	213	72	216	101	72	214	100	72
29[b]	215	68	219	101	69	213	99	69
33	224	68	227	101	69	221	99	69
38	233	68	237	102	69	229	98	69
41	239	68	242	101	69	235	98	69
45	248	68	251	101	69	242	98	69
49[b]	256	65	259	101	66	252	98	66
53	266	65	270	102	66	260	98	66
57	276	62	277	101	66	269	98	65
61	285	62	288	101	66	276	97	65
65	290	61	288	100	66	277	96	65
69	296	61	292	99	66	281	95	65
73	300	61	295	98	64	284	95	65
77	303	61	297	98	62	284	94	64
81[b]	300	57	301	100	55	283	94	59
85	306	54	302	99	55	283	93	57
89	307	52	305	99	55	287	94	53
93	307	49	305	99	53	286	93	49
97	303	46	304	100	50	281	93	43
101	291	44	296	102	47	271	93	39
105[b]	288	37	295	103	43	271	94	33
109	290	32	288	99	28	273	94	26
113	289	24	273	94	24	260	90	23
117	283	18	264	93	18	256	90	21
121	277	13	264	95	14	231	84	13
123	268	13	260	97	13	231	86	10
Terminal sacrifice		12			13			9
Mean for weeks								
1-13	156		159	102		156	100	
14-52	225		229	102		223	99	
53-123	291		288	99		271	93	

[a] The number of animals weighed for this week is fewer than the number of animals surviving.
[b] Interim evaluations occurred during weeks 27, 47, 79, and 105.

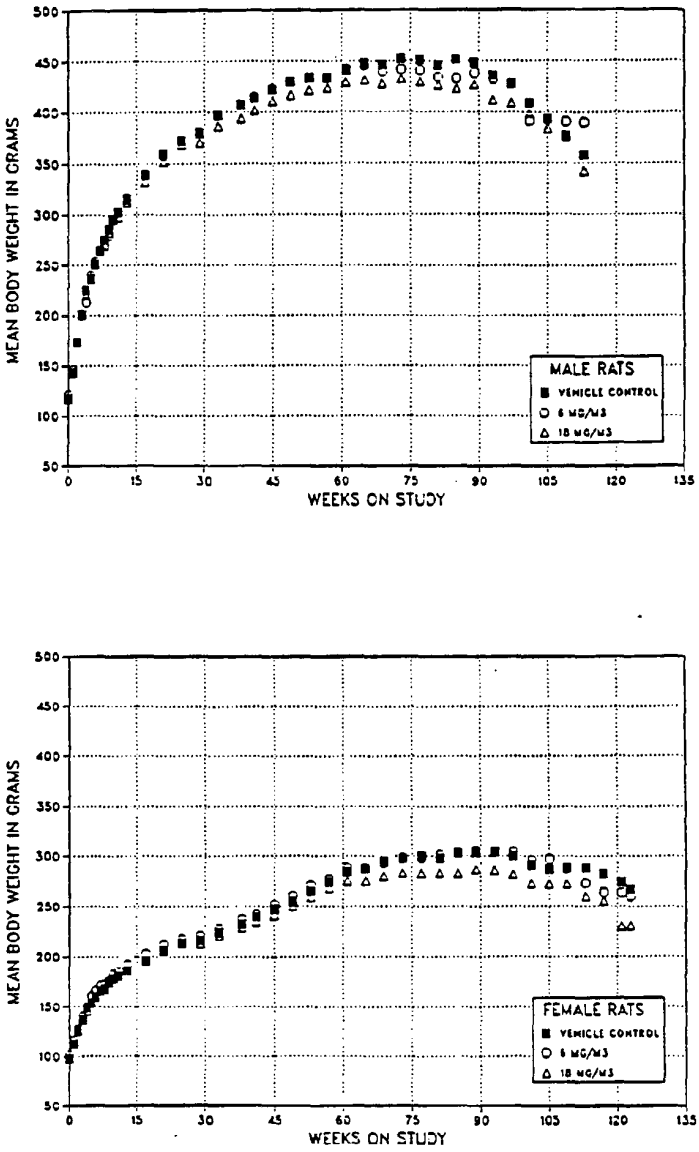

FIGURE 2
Growth Curves for Male and Female Rats Administered Talc by Inhalation for Their Lifetime

Lung: Absolute and relative lung weights of male rats exposed to 18 mg/m^3 were significantly greater than those of controls at the 6-, 11-, and 18-month interim evaluations and at the end of the study, while those of female rats exposed to 18 mg/m^3 were significantly greater than those of controls at the 11-, 18-, and 24-month interim evaluations and at the end of the study (Appendix E). Although lung weights of males exposed to 6 mg/m^3 were not significantly different from controls at any of the interim evaluations, those of females at the 18-month interim evaluation and at the end of the lifetime study were significantly greater.

Pulmonary lesions in male and female rats occurring in response to the inhalation of talc aerosols were generally similar at the interim evaluations and the end of the study, but varied in incidence, extent, and severity with exposure concentration and duration (Table 5). At necropsy, the lungs of exposed rats had multiple small, round, pale white lesions visible through the visceral pleura. These lesions were generally larger and more extensive in rats exposed to 18 mg/m^3 than in those exposed to 6 mg/m^3, and at the end of the study than at the earlier interim evaluations.

At the 6-month interim evaluation, the pulmonary lesions consisted of multiple, focal accumulations of alveolar macrophages and infrequent neutrophils within alveolar lumens (inflammation, granulomatous). When viewed under polarized light, the cytoplasm of the alveolar macrophages contained birefringent particles believed to be talc. In two female rats, the alveolar epithelium in some affected areas had increased numbers of low cuboidal type II pneumocytes (alveolar epithelial hyperplasia), but there was no apparent increase in the amount of collagen within the alveolar septa. The peribronchial lymphoid aggregates of several rats also contained focal accumulations of macrophages that varied from a few to approximately 10 cells in the plane of section (peribronchial hyperplasia, histiocytic).

In contrast to the first interim evaluation, hyperplasia of type II pneumocytes was associated with the intra-alveolar accumulations of macrophages in all exposed rats examined at 11 months. Moreover, in the most severely affected foci, the alveolar septa were thickened by the accumulation of reticulin and collagen fibers (interstitial fibrosis). The lesions in rats examined at 18 and 24 months and in core study rats were similar but generally larger and more extensive (Plates 1 and 2). Although alveolar macrophages predominated in the inflammatory lesions, varying numbers of neutrophils were also present and the interstitium contained infiltrates of mononuclear inflammatory cells (lymphocytes and macrophages). Moreover, epithelioid macrophages and multinucleated giant cells were also seen within foci of inflammation at these later time points. In some rats, there were well-delineated areas of fibrosis that completely obliterated the alveoli (Plates 3 and 4). Hyperplasia of the alveolar epithelium was often prominent at the margins of these lesions. The affected cells were cuboidal or columnar with prominent nucleoli and exhibited some pleomorphism.

In addition to the changes described above, squamous metaplasia of the alveolar epithelium (Plate 5) was observed in two male and eight female rats in the 18 mg/m^3 groups of the core study (Table 5). The metaplasia was usually associated with inflammation and was characterized by the replacement of alveolar type I and type II pneumocytes by well-differentiated keratinized squamous cells. Squamous cysts were also seen in three males and seven females in the 18 mg/m^3 groups and in one female in the 6 mg/m^3 group. The cysts had outer walls of well-differentiated, stratified squamous epithelium without cellular atypia and central lumens often containing sloughed keratin.

Although an alveolar/bronchiolar adenoma was seen in one 6 mg/m^3 female at the 18-month interim evaluation, the remainder of the pulmonary neoplasms were seen in rats in the core study (Table 6). The incidences of alveolar/bronchiolar adenoma, carcinoma, and adenoma or carcinoma (combined) in female rats exposed to 18 mg/m^3 were significantly greater than those of controls. A squamous cell carcinoma was also observed in an 18 mg/m^3 female. Alveolar/bronchiolar neoplasms occurred in two males exposed to talc aerosols, one at each of the exposure concentrations, and none were seen in control males. Because of the low number of affected male rats, these neoplasms could not be attributed to talc exposure.

Because of the moderate to marked hyperplasia of the alveolar epithelium associated with the inflammatory lesions and because of the fibrosis and

TABLE 5
Incidences of Selected Lung Lesions in Rats in the Lifetime Inhalation Study of Talc

	Male			Female		
	0 mg/m³	6 mg/m³	18 mg/m³	0 mg/m³	6 mg/m³	18 mg/m³
6-Month Interim Evaluation						
Lung[a]	3	3	3	3	3	3
Inflammation, Granulomatous[b]	0	3*(1.3)[c]	3*(2.3)	0	3*(1.3)	3*(3.0)
Peribronchial Hyperplasia, Histiocytic	0	1 (1.0)	2 (2.0)	0	1 (1.0)	2 (1.0)
Hyperplasia, Alveolar Epithelium	0	0	0	0	1 (1.0)	1 (1.0)
11-Month Interim Evaluation						
Lung	2	3	3	3	3	3
Inflammation, Granulomatous	0	3*(1.7)	3*(3.0)	0	3*(1.7)	3*(2.7)
Peribronchial Hyperplasia, Histiocytic	0	0	0	0	1 (1.0)	2 (1.5)
Hyperplasia, Alveolar Epithelium	0	3*(2.0)	3*(1.7)	0	3*(1.0)	3*(2.3)
Interstitium, Fibrosis, Focal	0	2 (1.0)	3*(1.0)	0	2 (1.0)	3*(1.0)
18-Month Interim Evaluation						
Lung	3	3	2	3	3	3
Inflammation, Granulomatous	1 (1.0)	3 (1.3)	2 (2.0)	0	3*(1.7)	3*(2.0)
Peribronchial Hyperplasia, Histiocytic	0	2 (1.0)	2 (1.0)	0	1 (1.0)	2 (1.0)
Hyperplasia, Alveolar Epithelium	1 (1.0)	3 (1.0)	2 (1.0)	1 (1.0)	3 (1.0)	3 (1.3)
Interstitium, Fibrosis, Focal	0	3*(1.0)	2 (1.5)	0	3*(1.3)	3*(1.7)
Alveolar/bronchiolar Adenoma	0	0	0	0	1	0
24-Month Interim Evaluation						
Lung	3	6	2	5	9	3
Inflammation, Granulomatous	0	6*(1.5)	2 (2.0)	1 (1.0)	9**(1.4)	3 (1.7)
Peribronchial Hyperplasia, Histiocytic	0	1 (1.0)	1 (2.0)	0	2 (1.0)	0
Hyperplasia, Alveolar Epithelium	0	6*(1.0)	2 (1.5)	1 (1.0)	9**(1.4)	2 (2.3)
Interstitium, Fibrosis, Focal	0	5*(1.0)	2 (1.5)	0	8**(1.4)	3*(3.0)
Core Study						
Lung	49	50	50	50	48	50
Inflammation, Granulomatous	2 (1.0)	50**(1.6)	49**(2.3)	2 (1.5)	47**(1.5)	50**(2.8)
Peribronchial Hyperplasia, Histiocytic	0	12**(1.3)	8**(1.9)	0	8**(1.3)	9**(1.3)
Alveolar Epithelium, Hyperplasia	5 (2.0)	26**(1.3)	38**(1.7)	2 (1.0)	27**(1.2)	47**(2.1)
Alveolus, Metaplasia, Squamous	0	0	2 (1.0)	0	0	8**(1.1)
Interstitium, Fibrosis, Focal	1 (1.0)	16**(1.2)	33**(1.8)	1 (1.0)	24**(1.5)	44**(2.1)
Cyst (Squamous)	0	0	3	0	1	7**

* Significantly different (P≤0.05) from the control by Fisher's exact test (interim evaluation) or logistic regression (lifetime study)
** P≤0.01
[a] Number of animals with lung examined microscopically.
[b] Number of animals with lesion.
[c] Average severity grades of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

TABLE 6
Incidences of Lung Neoplasms in Rats in the Lifetime Inhalation Study of Talc

	Male			Female		
	0 mg/m³	6 mg/m³	18 mg/m³	0 mg/m³	6 mg/m³	18 mg/m³
Core Study						
Alveolar/bronchiolar Adenoma						
Overall rates[a]	0/49 (0%)	1/50 (2%)	1/50 (2%)	1/50 (2%)	0/48 (0%)	9/50 (18%)
Terminal rates[b]	0/9 (0%)	0/14 (0%)	1/16 (6%)	0/11 (0%)	0/13 (0%)	1/9 (11%)
First incidence (days)	–[d]	781	799 (T)	805	–	716
Logistic regression[c]	P=0.494	P=0.527	P=0.615	P<0.001	P=0.503N	P=0.010
Alveolar/bronchiolar Carcinoma						
Overall rates	0/49 (0%)	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/48 (0%)	5/50 (10%)
Terminal rates	0/9 (0%)	0/14 (0%)	1/16 (6%)	0/11 (0%)	0/13 (0%)	3/9 (33%)
First incidence (days)	–	–	799 (T)	–	–	828
Logistic regression	P=0.370	–[e]	P=0.615	P=0.003	–	P=0.028
Alveolar/bronchiolar Adenoma or Carcinoma						
Overall rates	0/49 (0%)	1/50 (2%)	1/50 (2%)	1/50 (2%)	0/48 (0%)	13/50 (26%)
Terminal rates	0/9 (0%)	0/14 (0%)	1/16 (6%)	0/11 (0%)	0/13 (0%)	4/9 (44%)
First incidence (days)	–	781	799 (T)	805	–	716
Logistic regression	P=0.494	P=0.527	P=0.615	P<0.001	P=0.503N	P<0.001
Squamous Cell Carcinoma						
Overall rates	0/49 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/48 (0%)	1/50 (2%)

(T) Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined microscopically.
[b] Observed incidence at terminal kill
[c] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard these lesions as nonfatal. A lower incidence in a dose group in indicated by N.
[d] Not applicable; no tumors in animal group
[e] Value of statistic cannot be computed.

inflammation occurring within some of the neoplasms, there was considerable difficulty in determining the biological nature of the proliferative lesions observed and in distinguishing hyperplasia from adenoma and adenoma from carcinoma. The adenomas were irregular, circumscribed masses consisting of cuboidal to columnar epithelium arranged in alveolar, tubular, or papillary formations and separated by varying amounts of collagenous connective tissue. The neoplastic epithelium generally formed a single layer and was relatively uniform with minimal cellular atypia. The carcinomas were distinguished from the adenomas on the basis of having greater cellular pleomorphism and atypia, but they exhibited little evidence of invasion and none metastasized (Plates 6 and 7). In several benign and malignant neoplasms, the central portion of the mass was composed primarily of dense collagen and the epithelial component was located at the periphery. The extent of fibrosis in these neoplasms is not typical of spontaneous alveolar/bronchiolar neoplasms in control F344/N rats. The fibrous connective tissue was not interpreted as being a primary scirrhous response to the neoplastic epithelium, but rather a component of the prolonged inflammatory reaction to talc.

Lymph node: Histiocytic hyperplasia, consisting of accumulations of macrophages in the subscapular and medullary sinuses, occurred in the bronchial lymph nodes (male: 0 mg/m³, 0/41; 6 mg/m³, 44/48; 18 mg/m³, 46/49; female: 0/46, 40/47, 45/47) and in the mediastinal lymph nodes (male: 0/48, 40/49, 43/47; female: 0/47, 33/44, 40/47) of rats exposed to talc (Tables A4 and B4). The macrophages had foamy cytoplasm filled with birefringent particles of talc.

Nose: Hyperplasia of the respiratory epithelium of the nasal mucosa occurred in three male rats exposed to 6 mg/m^3 and 14 male rats exposed to 18 mg/m^3, but not in the control group (Table A4). The lesion consisted of an increase in the number of goblet cells primarily in the mucosa of the nasal septum. Hyperplasia of the respiratory epithelium also occurred in several female rats, but the incidences in the exposed groups were not significantly increased (Table B4).

During the pathology review process, it was noted that male and female rats in control and exposed groups had large eosinophilic droplets in the cytoplasm of the olfactory and, to a lesser extent, the respiratory epithelium. The lesion (cytoplasmic alteration) was focal or multifocal and usually located near the junction of the two epithelial types. Although present in the controls, the incidences were increased in exposed rats (males: 3/49, 18/48, 40/47; females: 5/48, 23/45, 46/48).

Adrenal medulla: Focal adrenal medulla hyperplasia or pheochromocytoma were observed in rats at the various interim evaluations, but the number of affected rats was too small to draw definitive conclusions. However, in the core study, benign, malignant, or complex (combined) pheochromocytomas occurred with a significant positive trend in male and female rats, and the incidences in the 18 mg/m^3 groups were significantly greater than those of controls by pairwise comparisons (Table 7). Moreover, bilateral pheochromocytomas were more frequent in exposed male rats than in controls (Tables A3 and B3). Although adrenal medulla hyperplasia occurred with similar frequency among exposed and control female rats, the incidences of hyperplasia in exposed males were significantly lower than controls. The lower incidences in exposed males are possibly due, in part, to the reduced amount of normal medullary tissue (e.g., medullary tissue without a pheochromocytoma) in which to observe hyperplasia.

Focal hyperplasia and pheochromocytoma constitute a morphological continuum. Focal hyperplasia consisted of irregular, small foci of small to normal sized medullary cells arranged in packets or solid clusters slightly larger than normal; compression of the surrounding tissue was minimal or absent. Pheochromocytomas were generally larger than focal hyperplasia, caused variable compression of the surrounding parenchyma, and many obscured much or all of any remaining normal medullary tissue. The neoplastic cells were arranged in variably sized aggregates, large solid clusters, and/or trabecular cords several layers thick separated by a delicate fibrovascular stroma. The larger neoplasms usually exhibited greater cellular pleomorphism and atypia than smaller neoplasms. Because the only morphological criteria that unambiguously distinguish malignant from benign pheochromocytomas is frank invasion or metastasis, a diagnosis of malignant pheochromocytoma was made only when there was invasion of the capsule. Complex pheochromocytomas consisted of an admixture of neoplastic pheochromocytes and neuroblasts, ganglion cells, and/or Schwann cells.

Lung Talc Burden

The lung talc burdens of exposed rats, normalized to control lung weight or exposure level, are presented in Tables F2 and F3. The lung talc burden normalized to control lung weight (mg talc/g control lung) adjusts for differences in lung weight between sexes or at different ages. The lung burden normalized to control lung weight and exposure level (mg talc/g control lung/mg/m^3) adjusts for exposure level to determine the effect of exposure concentration on talc clearance from the lung.

The data, normalized to control lung weight, show that talc burdens of rats exposed to 6 mg/m^3 were similar between males and females and increased progressively from 6 to 24 months (Table F2). Lung talc burdens in females exposed to 18 mg/m^3 also increased progressively from 6 to 24 months. In contrast, lung talc burdens of males at the 18 mg/m^3 exposure concentration increased from 6 to 18 months, but remained about the same at 18 and 24 months.

The exposure-normalized data show that lung talc burdens were generally proportional to exposure concentration at each interim evaluation. The exposure-normalized lung burdens of rats exposed to 6 or 18 mg/m^3 were generally similar at each of the interim evaluations except for slight increases for males at 6 and 11 months and females at 6 months (Table F3). This suggests that either clearance of talc was not substantially impaired by increasing the exposure concentration, or that clearance of talc was impaired similarly at both exposure levels.

TABLE 7
Incidences of Nonneoplastic Lesions and Neoplasms of the Adrenal Medulla in Rats in the Lifetime Inhalation Study of Talc

	Male			Female		
	0 mg/m³	6 mg/m³	18 mg/m³	0 mg/m³	6 mg/m³	18 mg/m³
11-Month Interim Evaluation						
Adrenal Medulla[a]	2	3	3	3	3	3
Hyperplasia[b]	0	0	0	0	0	0
Pheochromocytoma, Benign	1	0	0	0	0	0
18-Month Interim Evaluation						
Adrenal Medulla	3	3	2	2	3	3
Hyperplasia	0	1 (1.0)[c]	0	0	1 (2.0)	1 (2.0)
Pheochromocytoma, Benign	0	0	1	0	0	0
24-Month Interim Evaluation						
Adrenal Medulla	3	6	2	5	9	3
Hyperplasia	2 (1.5)	2 (2.0)	0	3 (2.0)	0	0
Pheochromocytoma, Benign	0	2	0	0	4	0
Pheochromocytoma, Benign, Bilateral	1	1	2	0	1	3
Core Study						
Adrenal Medulla	49	48	47	48	47	49
Hyperplasia	20 (2.7)	8**(2.3)	9*(3.2)	22 (2.5)	20 (2.2)	16 (2.6)
Pheochromocytoma, Benign						
Overall rates[d]	25/49 (51%)	30/48 (63%)	36/47 (77%)	13/48 (27%)	14/47 (30%)	18/49 (37%)
Terminal rates[e]	6/9 (67%)	11/14 (79%)	16/16 (100%)	5/11 (45%)	5/13 (38%)	6/9 (67%)
First incidence (days)	429	558	614	678	705	697
Logistic regression test[f]	P=0.007	P=0.213	P=0.009	P=0.185	P=0.541	P=0.225
Pheochromocytoma, Malignant						
Overall rates	3/49 (6%)	3/48 (6%)	7/47 (15%)	0/48 (0%)	1/47 (2%)	10/49 (20%)
Terminal rates	1/9 (11%)	1/14 (7%)	3/16 (19%)	0/11 (0%)	0/13 (0%)	3/9 (33%)
First incidence (days)	670	544	645	-[g]	849	784
Logistic regression test	P=0.096	P=0.662	P=0.178	P<0.001	P=0.509	P=0.001
Pheochromocytoma, Complex						
Overall rates	0/49 (0%)	2/48 (4%)	1/47 (2%)	0/48 (0%)	0/47 (0%)	0/49 (0%)
Terminal rates	0/9 (0%)	1/14 (7%)	0/16 (0%)	0/11 (0%)	0/13 (0%)	0/9 (0%)
First incidence (days)	-	558	743	-	-	-
Logistic regression test	P=0.486	P=0.230	P=0.503	-[h]	-	-
Pheochromocytoma, Benign, Malignant, or Complex						
Overall rates	26/49 (53%)	32/48 (67%)	37/47 (79%)	13/48 (27%)	14/47 (30%)	23/49 (47%)
Terminal rates	7/9 (78%)	12/14 (86%)	16/16 (100%)	5/11 (45%)	5/13 (38%)	8/9 (89%)
First incidence (days)	429	544	614	678	705	697
Logistic regression test	P=0.007	P=0.147	P=0.006	P=0.014	P=0.541	P=0.024

* Significantly different (P≤0.05) from the control by logistic regression
** P≤0.01
[a] Number of animals with adrenal medulla examined microscopically.
[b] Number of animals with lesion.
[c] Average severity grades of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[d] Number of animals with neoplasm per number of rats with adrenal medulla examined microscopically.
[e] Observed incidence at terminal kill
[f] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard these lesions as nonfatal.
[g] Not applicable; no tumors in animal group
[h] Value of statistic cannot be computed.